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ON THE PHYSIOLOGY OF PARASITISM.

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ON THE PHYSIOLOGY OF PARASITISM.

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AN investigation, begun at the suggestion of Professor V. H. Blackman and carried out under his general supervision, has been in progress for some years, having as its object a study of the physiological phenomena associated with fungal parasitism.[1] With the progress of the research, new branches have been opened, in many cases so inter-related that a definite pronouncement on any one cannot be given till others have been sufficiently explored. Thus publication has not kept pace with the actual progress of the investigation. Many results have been obtained, some which are so well established that they will shortly be published in final form; others which, though in themselves sufficiently established, cannot be dealt with fully until their exact relationships with other

[1] The following papers embodying certain results have been published from time to time in the "Annals of Botany" under the general title of "Studies in the Physiology of Parasitism."

I. W. Brown; "The Action of *Botrytis cinerea*." Ann. Bot., Vol. XXIX, 1915, p. 313.

II. V. H. Blackman and E. J. Welsford; "Infection by *Botrytis cinerea*." Ann. Bot., Vol. XXX, 1916, p. 389.

III. W. Brown; "On the Relation between the Infection Drop and the underlying Host Tissue." Ann. Bot., Vol. XXX, 1916, p. 399.

IV. W. Brown; "On the Distribution of Cytase in Cultures of *Botrytis cinerea*." (In the press) Ann. Bot., Vol. XXXI, 1917.

phenomena, not as yet fully investigated, have been determined; others again which merely serve at present as starting points for further work. It has been thought advisable therefore to publish the following account, which aims at being a résumé of the features already elicited, both published and unpublished, together with a general forecast of the lines which further investigation of the subject may be expected to follow. The present somewhat informal publication has the personal advantage of affording the writer an opportunity to express certain views which appear to him plausible in the light of present knowledge but which may require to be modified as knowledge of the subject extends. It is hoped that it will also enable the general botanical reader to form a more connected picture of the idea and progress of the investigation than may be obtained from the detailed narratives which have appeared and are appearing from time to time elsewhere.

Botrytis cinerea, the fungus which has formed the chief object of study, is a member of that large group of parasitic fungi which are characterised by the fact that they kill the tissue of the host plant in advance of their growth. This species was selected for the present work on account of its ubiquity, the readiness with which it reproduces itself, the rapidity and certainty with which its spores germinate, and the strongly marked features of its parasitic attack. This species is of further interest in that the majority of investigations dealing with the subject have centred round it or near allies.

It is not proposed to deal in detail with the statements in the literature bearing on this question.[1] Beyond the generally attested fact that such fungi kill in advance of their growth, much of the experimental evidence is of a highly unsatisfactory nature, and the statements put forward by various writers are in many cases contradictory. The primary object of the research was to aim at deciding on certain of these controversial points, and in the first instance to examine the nature of the principle of the fungus which is responsible for the phenomenon of "action in advance." This phenomenon has been known since the time of de Bary (1) to consist essentially of two processes, (a) the solution of the cell wall or at least of certain of its constituents so that the tissue loses its coherence, (b) the killing of the living protoplasmic contents themselves. De Bary believed the phenomenon to be due chiefly to the action of an enzyme, but considered that certain salts (oxalates)

[1] For a detailed criticism, see No. I of the series already cited.

excreted by the fungus played a part in the killing action. Later investigators confirmed the presence of an enzyme in connection with the action and little doubt remained that the action manifested on the cell wall of the host was due to this substance. As regards the killing action of the fungus on the host cell, there has been a progression from the view of de Bary, according to which soluble oxalates may play a part in the effect, to that of Behrens (2), who states that the toxic action is not due to a substance of volatile or enzymatic nature, and finally to that of Smith (3), who ascribes the whole toxic action of the fungus to soluble oxalates.

From a perusal of the literature, it appeared that previous investigators had employed, for the study of the active principle concerned, extracts obtained from comparatively old cultures or from host tissue which had been parasitised by the fungus. Now the actively invading portion of the fungus is essentially of the nature of a young and fresh culture. It may therefore be objected to the former method that extracts of old mycelia do not necessarily bear any close relationship to those of a young vigorous culture, and that in particular the former extracts would contain various products such as the so-called "staling" products which would affect the experiments *in vitro* while they would play no direct part in the phenomenon of parasitic attack. To the extracts obtained from parasitised host tissue, there is, in addition to the foregoing, the further objection that such extracts contain substances derived from the host and not from the fungus at all. It was argued that the presence of the latter would render the experimental results difficult of interpretation, if not entirely nugatory.

I. Nature of Active Principle of Fungus.

Preparation of Extract. The aim of the investigation was thus to prepare in the first place an extract from young hyphæ alone, and it was hoped that this extract might prove to be of a sufficiently powerful nature to recommend it for use in this connection. The method which was finally adopted for this purpose is described in detail elsewhere. It consisted essentially in the sowing of a large quantity of spores in a suitable nutrient on horizontal glass plates. A short germination period (ca. 24 hours) was allowed, after which the spores were washed, dried, and ground to a fine powder. The latter was extracted in water, and a clear extract obtained by centrifuging off the débris.

Action of Extract on Plant Tissues.

The method of experiment consisted simply in placing pieces of plant tissue in the extract. In the case of leaves and floral structures, it was found advisable to inject with the extract, as otherwise the action only proceeded slowly from the cut surface inwards (see later). Experiments in which the leaves, etc., were injected with water served to control such injurious effects as might be produced by the process of injection.

In all cases the action of the extract was found to be of a two-fold nature: (1) An action upon the cell walls of the plant, resulting in the complete disorganisation of the cell wall structure, so that the cells separate readily from each other and the cellulose envelope of each individual cell becomes disintegrated; (2) an action upon the living cell itself, resulting in its death.

The former action is shown by the tissue losing its coherence. Thus after a short treatment (about half an hour) with the extract, discs of potato or turnip tissue ($\frac{1}{2}$ mm. thick) readily fall to pieces on handling. A similar rotting effect is produced in the case of many foliar and floral structures. In the case of some petals the action is extremely rapid, the injected part becoming limp and obviously disorganised within a few minutes of injection.

A method of quantitatively determining the activity of the extracts employed was elaborated, having as its basis the determination of the time necessary for the decomposition of discs of tissue (potato, turnip) of standard thickness.

The killing action of the extract is shown variously in different cases. In the case of coloured structures it is shown by the escape of the colouring substance from the cells on death. Thus petals of rose, *Viola*, *Gloxinia*, etc., on treatment with the extract rapidly become decolorised. In other cases death of the cells is shown by the development of a colouration which is held in abeyance as long as the cells are alive. Thus when leaves of broad bean are injected with extract, they rapidly become black; when lettuce leaves are similarly treated they become brown. These latter phenomena are due to oxidase reactions which are particular to the plants concerned and have no relation to the fungal extract as such. In the case of colourless tissues, the killing action of the extract can be followed by examining the relation of the affected cells to plasmolysis in hypertonic solutions.

The action of the extract has been tested in this way on a

large number of plant structures. The following general conclusions are drawn:—

Fleshy structures are very generally susceptible to the action of the extract. Among such have been tested the tissue of potato, swede, turnip, radish, apple, cucumber and a number of succulents, (species of stonecrop, *Cotyledon*, etc.).

Petals and floral structures generally are rapidly destroyed. Upwards of thirty species of plants have been tested in this respect, and in all cases a rapid destructive action on the part of the extract has been established. It is highly probable that this behaviour is characteristic of floral structures generally.

Among ordinary foliage leaves a strong action of the extract could be established in some cases, *e.g.*, leaves of broad bean, lettuce, violet, *Petunia*, various speciesof *Begonia*, etc. In other cases the action was of a much less marked character; with leaves of a eathery or woody texture no definite action could be established in any case.

The extract so far as could be seen had no action whatever on tissues of mosses or liverworts. It was also without action on filaments of algæ and on bacteria.

The detailed features of the action of the extract were followed out in the case of certain tissues (potato, turnip, cucumber, etc.), the plasmolytic criterion of death being here employed. It was shown that the first demonstrable action consisted in the solution of the middle lamella uniting contiguous cells, with the result that coherence of the tissue was destroyed. The attack was, however, not confined to this, and the general body of the cell disintegrated, though complete solution of all constituents did not take place. At a comparatively late stage in the process the cells lost their power of becoming plasmolysed in hypertonic solutions. Thus, of the two manifestations of the action of the extract, the toxic is subsequent to the enzymic.

In dealing with the action of the fungal extract upon tissues, special attention was directed to the post-mortem features evinced. It was found in all cases that these were identical with those induced by the action of the fungus itself. Again, in all cases where a distinct parasitism of the fungus on a particular host could be established, it was found that the tissues of the latter were acted upon in a similar way by the fungal extract; and where the extract was without action (*e.g.* mosses and liverworts), no definite action could be established on the part of the fungus itself. These con-

siderations justify the conclusion that the extract in question is a true representation in essentials of the active principle of the fungus and that therefore the effects of the fungus can be explained on the basis of the properties of the fungal extract.

Behaviour of Fungal Extract to Reagents. An examination of the effect of various substances upon the activity of the extract was carried out. The main results arrived at were as follows:—

Acids and Alkalies. The extract shows a slight acid reaction. When this is slowly diminished by careful addition of alkali, the activity of the extract diminishes, and finally becomes *nil* in the neighbourhood of the neutral point. In alkaline solution the action is in all cases inhibited. On restoring the extract to its original faintly acid condition its activity is restored. With increasing concentrations of acid the activity of the extract again diminishes, the effect of mineral acids being in this respect more marked than that of organic acids. Thus the maximum activity of the extract is shown in faintly acid solution.

Salts. Addition of salts to the extract results in a diminution of activity. The specific retarding action varies with different salts, that of magnesium sulphate, for example, being much greater than that of potassium nitrate.

Substances such as cane sugar and glucose showed a slight retarding effect only when present in high concentration.

Plant Juices. In accordance with the above it was anticipated that plant juices would, on account of the presence in them of various acids, salts, etc., tend to retard the action of the extract. It was therefore of interest to determine how far the resistance of a particular tissue to the action of the extract (and of the fungus itself) could be correlated with the retarding action of its juice upon the activity of the extract. The juices of highly susceptible tissue, —*e.g.*, potato, turnip—and of highly resistent tissue, *e.g.*, liverwort, were compared in this respect. It was found that the retarding action of the latter was of the same dimensions as, and in no respect more marked than that of the former. The conclusion arrived at therefore was that the resistent power of a particular tissue to the action of the fungus (and fungal extract) was not directly related to the chemical nature of its cell sap.

Certain Physical Relationships of Extract.

The results here obtained may be summarised as follows:—

a. Relation to Heating. The activity of the extract, both as

regards its action on the cell walls and on the protoplasmic contents is totally destroyed by a sufficient degree of heating. Deactivation by heat is very rapid at 60°C. At 65°C it is as near as may be instantaneous.

b. Relation to Mechanical Shaking. The extract can be deactivated by agitation, *e.g.*, either by shaking in a closed vessel or by bubbling a stream of air (or nitrogen) through it. The effect is quite marked at ordinary temperatures (15-20°C), but increases very rapidly with rise in temperature.

c. Relation to Diffusion. The extract was submitted to diffusion through a series of graded gelatine membranes prepared in the first instance after the method of Bechhold (4) and later according to the writer's method (5). Dialysates containing various, concentrations of the active principle were thus obtained. All the dialysates which showed an action on the cell wall of tissue were also active in killing the cell contents.

From a series of comparative diffusion experiments it was shown that the active principle of the extract possessed a coefficient of diffusion through gelatine comparable with that of dextrin and somewhat greater than that of diastase.

Dialysis of crystalloidal contamination from the active principle was effected by the use of a certain type of collodion thimble (6), as well as by means of gelatine membranes of high gelatin content. The dialysate was found to show no action either on the cell walls of tissue or on the protoplasmic contents.

The dialysate was found to contain a small amount of a calcium salt in solution. The presence of a soluble oxalate in the extract, even in minimal quantity, is thus excluded.

Conclusions as to nature of Active Principle.

The action of the fungus and fungal extract on tissue has been seen to consist of an action on the cell walls together with an action on the living protoplasmic contents. These actions must be assumed *a priori* to be different in nature and to be brought about by different agents. For convenience of discussion we shall therefore speak of the "macerating" and "lethal" principles of the extract, these being responsible respectively for the macerating action (*i.e.*, action on the cell walls) and lethal action (*i.e.*, action on the living protoplasm) of the extract.

It has been shown in the foregoing that the macerating action of the fungal extract can be destroyed in various ways: by heat,

by mechanical agitation, and by neutralisation with alkali. The extracts so deactivated possess no lethal activity whatever. From microscopical investigation it is known that death of the cell takes place at a late stage in the process of disintegration of the cell walls. The latter process is therefore the determining factor of the whole action, that is, there is or is not lethal action according as there is or is not previous macerating action. This dependence of lethal upon macerating activity may be explained according to a variety of hypotheses.

(1) *That there is no distinct lethal substance but that death of the cell follows on account of the physiological derangement caused by the disorganisation of the cell wall.* This hypothesis is unlikely in view of the rapidity with which death of the cells takes place. It is known from plasmolytic studies that the association of cell wall and protoplasm can be broken for many hours without death of the cell ensuing. The rapid effects obtained in the present case cannot, therefore, be ascribed to this cause.

(2) *That the toxic principle is produced by action of the macerating principle on the cell wall, and does not exist, therefore, in the initial extract.* This view must be abandoned in the face of experimental evidence that extract in which a considerable quantity of cell wall has been digested behaves identically in all respects with the original extract.

(3) *That the two actions are due to different substances, but the lethal substance is unable to reach the protoplast until the permeability of the cell wall has been sufficiently increased by the action of the macerating substance.* If there be a lethal as distinct from a macerating substance, it must possess the following characteristics. From the diffusion experiments with gelatine, above mentioned, it must possess a diffusive capacity at least equal to that of the macerating substance; also it cannot be crystalloidal, and in particular it cannot be a soluble oxalate. From the data regarding extracts which have been deactivated in various degrees by heat, mechanical agitation, neutralisation with alkali, it is clear that the lethal substance, if present, must remain in the deactivated extracts (it cannot be assumed to show exactly the same sensitiveness in these respects as the macerating substance). The lethal principle must thus be assumed to be present in deactivated extracts, though it cannot act in virtue of its inability to diffuse through the unaltered cell wall. Killing of the cells should then continue independently of the macerating action after a certain stage is reached, that is when the

permeability of the cell wall has been sufficiently increased. Nevertheless it was found that if the macerating action was stopped (by addition of the requisite amount of alkali), even at a very late stage, the killing effect is strongly retarded. Such an experimental result is not favourable to the present hypothesis.

(4) *That both actions are due to the same substance or group of substances.* This view appears to the writer to be the simplest, and so far as is known, is in agreement with all the experimental facts.

While hypotheses (1) and (2) are in the mind of the writer definitely excluded, a final decision between (3) and (4) cannot at present be given. From the point of view of the progress of the present investigation the question at issue is not of primary importance, as it is sufficient for present purposes that the lethal action of the extract has been shown to be indissolubly connected with its macerating action. On theoretical grounds, however, this question is of very considerable moment. If the last-mentioned hypothesis be confirmed it would appear that we have in the active principle of the fungal extract a protoplasmic toxin which is presumably of an enzymic (*i.e.*, hydrolytic) nature. It is known that the active principle under consideration shows considerable specificity in regard to its behaviour towards cell wall constituents (cell walls of different plants, different constituents of the same cell wall). If such a behaviour could be shown in the case of the corresponding protoplasmic membranes, a practicable study of protoplasmic specificity could be undertaken. The main obstacle to such a study is one of technique. It would be essential to study the action of the extract upon the protoplasmic membrane independently of previous action on the surrounding cell wall, that is it would be necessary to experiment with protoplasts which are naturally naked or with ones which have been made so artificially. As the latter would probably furnish the data of chief interest, the technical difficulties in the way of this investigation are obvious.

Conclusions on the nature of the Fungal Extract.

Whichever hypothesis be accepted as explaining the lethal action of the extract, it is clear in any case that the chemical nature of the cell wall is of fundamental importance in relation to the action of the fungal extract upon the cell. In all cases it has been found that if the cell wall is disintegrated, death of the cell ensues; if the cell wall is not affected, neither are the living contents

of the cell. In other words, the nature of the cell wall affords the key to the resistance of the particular tissue to the action of the fungal extract and therefore also of the fungus. In particular, certain experiments lead to the conclusion that there are important chemical differences between the cell walls of higher plants and those of lower forms, such as liverworts. These considerations point to the desirability of a more complete study of the hemicellulose (or pectin?) series of cell wall constituents than has yet been attempted.

General Conclusions on the nature of the Fungal Extract.

At this stage it will add to clearness to summarise the main results obtained.

The action of the fungus on plant tissue may be studied on the basis of the action of the extract employed. The macerating principle of the latter is of the natare of an enzyme and is a colloid of intermediate diffusive capacity. The lethal principle, if different from the former, is also colloidal, with a diffusive capacity at least as great as that of the enzyme. Crystalloids, and in particular, soluble oxalates play no part whatever in the lethal activities of the extract.

II. Excretion of Active Principle by the Fungus.

From the phenomenon of "action in advance" already alluded to, it is clear that the active principle of the fungus is able to diffuse out of the fungal hypha into the plant tissue. An investigation was carried out with the object of determining to what degree the fungus was able to excrete its active principle into the culture medium. The results of this investigation are chiefly of technical and enzymological interest, and as they are of subsidiary importance in the progress of the investigation except in so far as they serve to indicate how the various contradictory statements on the subject have arisen, they will merely be referred to here. The amount of enzyme present in the culture fluid was found to increase up to about the time at which active growth of the hyphæ ceased, that is, to the time at which "staling" of the culture was reached, after which, the concentration continuously diminished. The nature of the preparation obtained was found to depend also upon the density of sowing and on the nature of the nutrient fluid. Some of the preparations obtained in this way are of a very active nature and contain much higher concentrations of enzyme than the standard extract previously employed. They are, however, less suitable for such a study as that described in Section I of this paper on account

of the presence in them of considerable quantities of impurities derived from the culture fluid.

III. On the Entrance of the Fungus into the Host Tissue.

Fungi of the type of *Botrytis cinerea* are able, in favourable circumstances, to attack their host when simply brought in contact with the external surface of the latter. Their entrance is not conditional upon the existence of pre-formed injuries as is the case with so-called "wound" parasites, nor do their germ tubes as a rule enter by way of the stomata or other natural apertures, but penetrate directly through the epidermis. A study of the mechanism of this process of penetration is obviously of fundamental importance.

The dominant view on this question is that due to de Bary (l.c.). In the case of the allied fungus, *Sclerotinia Libertiana*, he stated that the fungus, when placed in contact with the epidermis, possessed the power of killing the underlying cells of the host before any actual penetration of the latter had taken place. In consequence of this an exosmosis of food material takes place on to the epidermis. This serves to promote the growth of the fungus which is now able to penetrate directly into the underlying cells.

In the light of the above view it was important to determine whether the extract employed in the present investigation was able to exert any influence when placed on the outside of the host plant. Such action would consist in the death and disintegration of the underlying epidermal cells, with or without an alteration of the mechanical properties of the cuticle.

These experiments were carried out in very great detail and on a considerable number of plants. Though in a very few cases traces of action by the fungal extract under these conditions could not altogether be discounted, the evidence obtained was overwhelmingly in favour of the opposite view. In the great majority of cases no action whatever on the part of the extract when laid on the surface of plant structures could be demonstrated. The striking nature of this feature may be illustrated by mention of a particular experiment. A fungal extract when laid on a rose petal for twenty-four hours produced no change whatever. After this lapse of time, injection of the same liquid into the same petal produced complete disintegration within half an hour.

It was thus clear that the extract under consideration is unable to penetrate cuticularised tissue and thus cannot explain the killing-before-penetration effect described by de Bary. It was necessary

therefore to examine the early stages of infection by the actual fungus in order to check the statements of de Bary.

De Bary's view necessitates the existence of a toxic substance capable of diffusing through cuticularised membrane. This consideration postulates a considerable diffusive capacity on the part of this substance so that it should become uniformly distributed (or nearly so) throughout the limits of the small drop of nutrient in which the fungal spores are placed on the plant. Thus if such a toxic substance be present, the underlying tissue should be killed as near as may be simultaneously over the whole surface of contact of the drop and the plant. In a large number of cases however it was shown that the first appearances of attack showed a definite pattern within the drop, commencing as a marginal ring and spreading centre-wards. Again, on the same hypothesis, the time for the attack to develop should depend directly on the concentration of spores present, and inversely on the size of the nutrient drop, the larger the latter the longer the time required till an effective concentration of the toxic substance is reached. Nevertheless it was found that within very wide limits the time required for attack to manifest itself was independent of the number of spores present and of the size of the drop. Also, when drops in which infection had just been established were taken up, cleared of spores and tested on fresh pieces of plant tissue, no killing action whatever was demonstrable.

Further convincing evidence on this subject was obtained by use of a conductivity method. Drops of water with or without spores were laid on certain plant structures, and the electrical conductivity of the drops determined from time to time. In the case of both spore-free and spore-containing drops, a gradual and equal increase of conductivity was shown up to a stage when macroscopic discolouration of the underlying tissue in the latter case was observable. The conductivity in the latter case then increased with great rapidity, thus showing that a strong exosmosis was taking place from the host cells in virtue of the action exercised by the fungus. The drops in which this rapid exosmosis had begun when subjected to microscopic examination invariably showed penetration by the fungus to have already taken place. Indeed, in a number of fortunate cases, early stages of penetration could be demonstrated in drops in which the rapid rise of conductivity had not yet begun. Thus it is clear that the rapid exosmosis of soluble cell contents which is necessary according to the de Bary hypothesis to enable

penetration of the cuticle to take place, actually takes place only when penetration has been fully established.

A microscopic investigation (No. II of the series) carried out with the usual technique confirmed these results in every way. It was shown that in the case of the attack of the fungus on leaves of broad bean, the underlying tissue was wholly unaffected up to the time when penetration had actually taken place.

It is clear therefore that the de Bary hypothesis is untenable, at least in the present case, and that *fungi such as Botrytis cinerea exert no influence whatever upon the host tissue until the barrier offered by the outer cuticularised layers of the latter has been passed.*

The process of penetration is thus conceived as follows:— When spores of *Botrytis cinerea* are placed in a drop of water on the surface of a plant, the sources of energy required to enable them to penetrate the cuticular barrier consist simply of that stored within the spores themselves and of that which diffuses passively from the uninjured plant into the water drop. The phenomenon, therefore, falls to be investigated along three distinct lines: (1) An investigation of the germinating capacity of the fungal spores in pure water and in nutrients. (2) An investigation of the rate of escape of nutrient materials from plant tissue into water placed in contact with its outer surface. (3) An investigation of the physics of cuticular resistance on the one hand and of the power possessed by fungi of penetrating membranes on the other. An investigation of the existence and nature of tropic forces also calls for treatment in this connection. These investigations are still incomplete in detail, but enough work has been carried out to indicate the lines along which advance will be made.

(1) *On the germinating capacity of fungal spores in water and nutrients.*

The necessity for this investigation was only brought home at a comparatively late stage in the research, so that only a limited number of data are yet to hand. The object in view was in the first instance a purely practical one—*viz.*, to control the amount of spore germination for the general experimental work.

The method of experiment consisted in the preparation of spore suspensions in water hy means of filtration through fine muslin. The water was purified by repeated centrifuging and renewal until the conductivity of the suspension equalled that of distilled water

Means of estimating and regulating the density of spore suspension were also devised. The following results are well attested.

With any given lot of spores when sown on glass in pure water the percentage of germination is a function of the concentration of the spores, the higher the concentration of spores present the lower the relative and absolute amount of germination. Thus, with any particular lot the concentration can be increased so that germination is totally inhibited.

When the spores are sown in very dilute nutrient a similar phenomenon is observed, the concentration required to produce inhibition of germination being increased.

The percentage and vigour of germination of spores under a given set of circumstances depends also upon the nature of the spores. The main fact revealed in this connection is that age of spores diminishes capacity for germination. Thus the germination in water of spores from somewhat old cultures can be inhibited by a lower concentration of spores than is the case with spores from a young culture. This process might be described as one of "attenuation" by age. Other methods of attenuation can no doubt be devised—such as, perhaps, tbe maintainance of the spores under somewhat unfavourable physiological conditions. A thorough examination of these relationships is certain to be of great value in the elaboration of methods for the experimental treatment of the whole subject.

A method was also sketched by which the amount and vigour of germination under any circumstances could be controlled by subjecting the spores to the influence of varying percentages of carbon dioxide in the atmosphere. It was found that the effect of small amounts of nutrient could be neutralised by placing the fungus in the appropriate concentration of carbon dioxide. Thus while germination of a particular sample in pure water could be inhibited by an atmosphere containing 10% carbon dioxide, a pressure of 30% carbon dioxide was necessary to inhibit germination in a certain concentration of turnip extract.

The amount of germination in water can also be controlled by addition of very small amounts of alkaline carbonate. Evidence was also obtained that the effect of slight concentration could be neutralised by the addition of the requisite amount of neutral nutrient solution. The experimental possibilities of this method have not yet been investigated sufficiently fully to allow of further statement.

(2) *On the escape of nutrients from plant tissue into water placed in contact with its outer surface.*

Investigation in this connection has gone so far as to establish that drops of water when placed on the epidermis of plant structures do not remain as such but that substances diffuse out into the drops from the living tissue beneath. The drops may thus come to contain an appreciable amount of nutrient available for the germination of fungal spores in the drops. The method adopted consisted in the laying of drops of distilled water on leaf, flower, etc. structures for definite intervals, these drops being subsequently taken up and added to drops of a spore suspension in pure water. The effect on germination of the drops which had been on plant epidermis were compared with that brought about by drops of distilled water which had lain a similar time in contact with glass. By this means the escape of nutrient substances could readily be detected in the case of certain living plant structures. Considerable exosmosis of such nutrients was shown in the case of petals of *Cereus*, *Phyllocactus*, certain varieties of tulip; intermediate amounts in the case of sweet pea; and small traces only in the case of rose petals, bean leaves, etc. The investigation was also carried on by an conductivity method, with confirmatory results.

The exosmosis of nutrients from tissue could also be demonstrated in special ways. Thus it was found by the spore method that in *Cereus* petals a very considerable exosmosis took place, in *Lilium* petals, on the other hand, very slight exosmosis. It was found that the attack of *Lilium* petals by the fungus could be inhibited by a pressure of 15% carbon dioxide, whereas the latter required to be increased to a pressure of 30% to inhibit attack on *Cereus* petals.

The magnitude of the exosmosis of nutrients may also be measured by noting the difference between the times necessary to produce infection when the spores are sown in nutrient and when the spores are sown in water, the spores in the former case being sown in a concentration sufficient to inhibit germination on glass in pure water. With increasing exosmosis the time difference becomes less and less, and in the case of certain petals (e.g. *Cereus*) may become infinitesimal.

(3) *On the physics of cuticular resistance and the penetrating power of fungi.*

From the fact that the fungal extract has no effect whatever when laid on cuticle, and from evidence obtained in the microscopi-

cal investigation already alluded to, it is clear that the actual process of penetration of the cuticle takes place in a purely mechanical way. In investigating this problem several subsidiary problems arise. Mechanical pressure requires the existence of a fulcrum or attachment of the germ tube to the cuticle. The nature of this attachment, its presence or absence in different fungi requires investigation. Again, evidence has been brought forward that different fungi under the same conditions have different powers of mechanical penetration. A method was devised for testing the penetrative power of fungi. This consisted of the use of a series of gelatine membranes of graded hardness (5). It was found that a membrane of greater hardness was required to prevent the passage of *Botrytis* than was required in the case of *Rhizopus* or *Penicillium*. It is hoped when the details of this method are worked out that the penetrative powers of fungi can be catalogued on the basis of this graded series of gelatine membranes.

The question of penetration cannot satisfactorily be treated without consideration of the tropic factors which may come into play. The researches of Miyoshi (7 and 8) pointed to marked chemotropic influences in this connection, but these have been to a large extent discounted by the later work of Fulton (9). The recent work of Graves (10) has demonstrated the existence of a positive chemotropism exercised by cane sugar and, more especially, certain unknown constituents of turnip juice, together with that of a marked negative chemotropism of the fungus to its own excrete products. In the case of membrane penetration, positive chemotropic forces may conceivably play a part; negative chemotropism to the excrete products of its growth is physically impossible under the circumstances (If anything, there should, on the hypothesis of of such a force, be a tendency for the germ tubes to grow *away* from the epidermis into the spore-free region of the drop). While a positive chemotropism is thus not excluded, experiments have already shown that penetration of membranes may take place under circumstances which exclude such a factor. Fungi have been found to penetrate the following membranes:—

(1) Membranes of paraffin wax, which were shown by collateral test to be impenetrable to the simplest electrolytes.

(2) Plant epidermes which had been removed and repeatedly washed till all soluble substances had been removed.

(3) Gelatine membranes which had been soaked in exactly the same nutrient as the spores were sown in.

In all cases vigorous penetration took place provided the spores were in a condition to germinate.

The view which seems at present most likely to the writer is that the stimulation to penetration is a contact one. It is quite feasible of course that chemical factors may influence the nature and degree of this "thigmotropic" response. It is however premature to dogmatise on this subject as the amount of reliable data available is insufficient for the purpose of generalisation.

General Considerations on Plant Immunity and Resistance.

The general trend of this research has been to show that nothing short of a full physiological investigation of fungal metabolism on the one hand and of at least certain aspects of higher plant metabolism on the other hand must be undertaken if any insight into the problems of resistance and immunity is to be obtained. The extraordinary diversity of the observational data on this subject is sufficient to prove the hopelessness of trying to explain these phenomena on the basis of one or two features of the fungus on one hand and of the parasite on the other. The establishment of parasitism by a particular fungus on a particular plant follows upon a long series of physiological actions on the part of the fungus and simultaneous actions or consequent reactions on the part of the plant. With highly specialised "obligate parasites" the reactions between host and parasite are of a very recondite nature, and as yet there seems no possibility of subjecting them to experimental analysis. The application of the methods of the present research to such cases is thus problematical. In the case however of less specialised forms such as *Botrytis*, the reactions of host and parasite are comparatively simple in virtue of the phenomenon of "action in advance." On this account the host plant can be treated as a special kind of culture medium. Thus the problem entails simply the investigation of the growth of the parasite in a culture medium together with that of the enzymic process by which the living plant is converted into a dead culture medium. Nevertheless though the whole problem can be attacked on the lines of known methods, the phenomena presented are of a complex character, and as has been already emphasised a complete physiological investigation of a large number of processes must be carried out if any advancement is to be made.

A scheme of research adequate to the needs of the problem may best be devised by consideration of the successive stages by

which parasitism on a particular plant becomes established. When fungal spores come in contact with the plant surface, the first desideratum is a sufficiency of water to enable germination to take place. An investigation of hygrometric conditions is thus necessary as determining the presence of this water, and also as preventing at a later stage the spreading of the attack by the drying up of the part affected. In cases where the initial spore germination is dependent on dew formation an investigation of the speed of germination is of great importance. Granted a sufficiency of moisture for germination, the capacity of the particular spores to germinate in the drop falls for consideration. This problem as has been shown entails a two fold investigation, *viz.*, the capacity of the spores to germinate in water in virtue of energy stored within themselves, and the passive exosmosis of food materials into the drop from the underlying plant tissue. The next essential step in the process is that the fungus with the food material available to it should be able to penetrate the cuticle (or, in certain cases, should be able to advance as far as the neighbouring stomata and so into the interior of the plant). The treatment of this problem entails the examination of the intrinsic penetrative power of the fungus on the one hand, and of the mechanical resistance of the cuticle on the other, together with an estimation of possible tropic factors concerned. The difficulty in this regard is one simply of methods of experiment. Once penetration of the cuticle has taken place, the problem becomes simply an enzymological one. If parasitism of the type shown by *Botrytis cinerea* is to be established, the fungus must be able to kill the adjacent host cells, and continue to live in and progress through the dead remains. The problem is therefore one of the action of the fungal principle upon the particular living cells, and of the capacity of the fungus to live and excrete its active principle in the juice of the plant.

In the case of the so-called wound parasites (among which most bacteria would appear to fall) the problem presented is much simpler, as the problems which arise antecedent to the penetration of the cuticle do not come into consideration. Such parasites are thus to be investigated on the lines of the latter part of the scheme sketched above.

The investigation of a number of parasite and host types, according to the scheme advocated in the present paper, would prepare the way for a treatment on physiological lines of the phenomena of resistance and immunity.

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